Toxicology in Vitro 27 (2013) 641–650

Contents lists available at SciVerse ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Proposed model for in vitro interaction between fenitrothion and DNA, by using competitive fluorescence, ^{31}P NMR, ^{1}H NMR, FT-IR, CD and molecular modeling

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ARTICLE INFO

Article history:
Received 28 May 2012
Accepted 3 November 2012
Available online 12 November 2012

Keywords:
Fenitrothion
Partial intercalation
Circular dichroism
^{1}H NMR and ^{31}P NMR
ONIOM

ABSTRACT

In this work we proposed a model for in vitro interaction of fenitrothion (FEN) with calf thymus-DNA by combination of multispectroscopic and two dimensional molecular modeling (ONIOM) methods. The circular dichroism results showed that FEN changes the conformation of B-DNA and caused some changes to C-DNA form. The FT-IR results confirmed a partial intercalation between FEN and edges of all base pairs. The competitive fluorescence, using methylene blue as fluorescence probe, in the presence of increasing amounts of FEN, revealed that FEN is able to release the non-intercalated methylene blue from the DNA. The weak chemical shift and peak broadening of ^{1}H NMR spectrum of FEN in the presence of DNA confirmed a non-intercalation mode. The ^{31}P NMR showed that FEN interacts more with DNA via its $-NO_2$ moiety. The ONIOM, based on the hybridization of QM/MM (DFT, 6.31++G (d,p)/UFF) methodology, was also performed by Gaussian 2003 package. The results revealed that the interaction is base sequence dependent, and FEN interacts more with AT base sequences.

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1. Introduction

The increasing production and application of pesticides for agricultural purposes have caused a serious risk for human health. In this regards, the organophosphorus insecticides due to their widely usage, causing a major ecotoxicological hazard especially for humans and non-target animals and may be act as carcinogen, mutagen and tumor promoters. Organophosphorus insecticides have been reported to exert their primary pharmacological and toxicological effects through the inhibition of acetyl cholinesterase (AChE) activity. Fenitrothion (O,O-dimethyl O-(3-methyl-4-nitrophenol) phosphorothiate) (FEN) is an organophosphorus insecticide (Fig. 1) which is extensively used in Iran for controlling of chewing and sucking insects, flies, mosquitoes and cockroaches. The enzyme, acetyl cholinesterase (AChE) is the major target of FEN. This insecticide acts by promoting phosphorylation of active site of AChE enzyme and inhibition of AChE activity, which causes neurotoxic effects due to the accumulation of the neurotransmitter acetylcholine in cholinergic synapses (Ricciardi et al., 2006; Damásio et al., 2007; Sebire et al., 2009). The toxicologic, cytotoxic and genotoxic effects of FEN on the male rats reproductive system (Struve et al., 2007); protein, ribose nucleic acid (RNA), deoxyribose nucleic acid (DNA) contents, RNA/DNA ratio of the fat tissue of silkworm (Surendra Nath et al., 1996); lipid peroxidation and antioxidant defense system of rat kidney (El-Demerdash, 2012); and DNA damage and erythrocyte micronucleus frequency of carp (*Cyprinus carpio* L.) fingerling (Sepici-Dincel et al., 2011) were previously reported. Farghaly and El-Maghraby in a study about toxicological effects of ^{14}C-fenitrothion on experimental animals demonstrated that, about 15% of administered ^{14}C-fenitrothion was distributed in liver, kidney, lung, fat, intestine, blood, heart, and brain (Farghaly and El-Maghraby, 2008). Also, it is well demonstrated that FEN is a suicide substrate for cytochrome P450. This compound undergoes oxidative desulfuration by P450 resulting in the release and subsequent binding of atomic sulfur to the enzyme. Consequently, the P450-dependent metabolism of certain endogenous substrates could be inhibited by exposure to this insecticide (Berger and Sultatos, 1997). It is demonstrated that in sufficient doses of FEN, typical cholinergic poisons, are produced. It also has cytotoxic effects on liver and lungs (Hayes and Laws, 1990) and causes a significant increase in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, creatinine and urea (Kadry et al., 2001). Histopathological examination of liver and kidney revealed a significant injury in rat after chronic fenitrothion exposure (Afshar et al., 2008). Today we know that the insecticides cause several types of cancers and DNA damaging (MacFarlane et al., 2010; Tope and Rogers,

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http://dx.doi.org/10.1016/j.tiv.2012.11.004

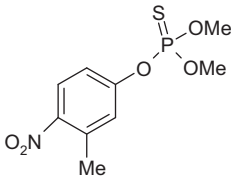

Fig. 1. The structure of fenitrothion.

2009; Zhang et al., 2010), therefore, the in vitro determination of mode of interaction and model of pesticides–DNA complex is the interest of many researchers (Patel et al., 2007; Axelrad et al., 2002). Recently, Roh and Choi (2011) studied on toxicological effects of FEN on immobility, growth, fertility, development, acetyl cholinesterase (AChE) activity and stress-response gene expressions of soil nematode (*Caenorhabditis elegans*). They suggested the cyp35A2 may be an important gene for exerting FEN toxicity in *C. elegans*. In addition, several reports demonstrated that the AChE enzyme resistance to the FEN. This is may be due to the mutations in the gene (ace) encoding of AChE enzyme by FEN (Fournier et al., 1992; Vaughan et al., 1997; Hsu et al., 2008; Weill et al., 2004). Hara et al. (1989) demonstrated a weak mutagenic effect for on *Salmonella typhimurium* TA100 that enhanced by the addition of S9 mix. In our previous work (Ahmadi and Jafari, 2011) we found out FEN may interact with DNA via partial-intercalation. In fact, only the changes in cyclic voltammetric of FEN at presence of CT-DNA and increase of intensity at λ_{max} accompanied by a red-shift of the λ_{max}, of UV–Vis spectrum of CT-DNA were considered as a sign to conclude that a partial-intercalation takes place. However, these data did not show exactly which atoms of FEN and CT-DNA participated at interaction. Therefore, it is of our interest to determine which atoms of FEN interact with DNA bases, ribose sugar and/or the backbone phosphate groups. In this work, a model for in vitro interaction of fenitrothion (FEN) with CT-DNA by combination of multispectroscopic techniques such as competitive fluorescence, circular dichroism (CD), ^{1}H NMR, ^{31}P NMR, FT-IR and molecular modeling method (ONIOM), in Tris–HCl buffer (0.01 M, pH 7.3) is proposed.

2. Materials and methods

Fenitrothion (45487) with high purity was kindly offered by Supelco (Sigma–Aldrich, Bellefonte, PA, USA). The solvent used for preparation of stock solution of FEN (1.0×10^{-2} M) was a mixture of H_2O–MeOH (30:70, %V:V). A serial dilution was used for preparation of other diluted solution of FEN from the above stock solution with Tris–HCl buffer (0.01 M, pH 7.3; in diluted solution the percentage of MeOH remarkably decreased >10%V:V and in this content of MeOH, the DNA did not deform (Kypr and Vorlíčková, 2002)). High polymerized calf thymus DNA was obtained from Supelco (Sigma–Aldrich, Bellefonte, PA). The stock solutions of CT-DNA were prepared by dissolving 2–5 mg of CT-DNA in 3.0 mL of Tris–HCl buffer solution at pH 7.3 and dialyzed exhaustively against the same buffer for 24 h. A solution of CT DNA gave a ratio of UV absorbance at 260 and 280 nm of above 1.8, indicating that the DNA was sufficiently free of proteins (Kashanian et al., 2007). The DNA concentration per nucleotide (or phosphate) was determined spectrophotometrically by using $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ (Ahmadi and Bakhshandeh-Saraskanrood, 2009). Stock solution of CT-DNA stored at 4 °C and not used after more than 5 days of storage. The solvent used for NMR study was a 5:1 mixture of D_2O (Merck, 99.8% D) and CD_3OD (Merck, 99.5% D). TSS (3-(trimethylsilyl)-1-propan sulfuric acid (Aldrich) was used as internal reference. The concentration of FEN solution was 2.0×10^{-4} M for NMR studies in the mixture of deuterated solvents.

2.1. Instrumentation

All fluorescence measurements were carried out with a Beckman spectrofluorometer (LS 45). Circular dichroism (CD) measurements were recorded on a JASCO (J-810) spectropolarimeter. Solutions of DNA and FEN were scanned in a 0.5 cm (1 mL) quartz cuvette. The spectra were recorded during progressive addition of FEN to the pure DNA solution. The FT-IR measurements were performed by Shimadzu (IR Prestige-21) instrument. The pH values of the solutions were adjusted by employing a Metrohm model 827 that uses a combined glass electrode. The ^{31}P NMR and ^{1}H NMR measurements were carried out with a Bruker Avance DPX 250 MHz (4.7 T).

2.2. Competitive fluorescence study

The competitive fluorescence spectroscopic experiments were carried out in a 1.0 cm path length cuvette at 25 °C. The excitation and emission slits were both 10 nm and the scan speed was 400 nm min^{-1}. The complex of DNA (5.0×10^{-5} M) and methylene blue (2.5×10^{-5} M) in Tris buffer (0.01 M, pH 7.3) was placed in a thermostated quartz fluorescence cuvette and titrated with the FEN solution. The total volume of the solution was 2.0 ml. After each addition (1.0 μL of 0.01 M FEN), the solution was mixed thoroughly and allowed to equilibrate thermally for 1.0 min prior to the fluorescence measurements. The fluorescence intensity was measured in the range of 650–730 nm with excitation wavelength at 610 nm.

2.3. FT-IR studies for interaction of DNA with FEN

The spectra of the DNA–FEN solutions were carried out by using a cell assembled with AgCl windows. Spectra were collected after interaction of different amounts of FEN with DNA solution (2.5×10^{-3} M) (r_i = [DNA]/[FEN] = 40, 4). The spectra were measured over the spectral range 4000–600 cm^{-1} with a nominal resolution of 2 cm^{-1} and a minimum of 50 scans. The blank windows, CO_2 and water were removed by BGK mode of instrument according to our previous work (Ahmadi and Jamali, 2012). The spectra were smoothed with Gollay procedure.

2.4. CD measurement

The CD studies were carried out in 0.01 M Tris–HCl solutions which contained 5.0×10^{-5} M of DNA. The spectra were recorded during progressive addition of FEN with $R'i = 1/r_i$ = [FEN]/[DNA] = 0.0, 0.3, 0.7 and 1.0. In the region of 200–350 nm the FEN has not a CD signal.

2.5. ^{31}P and ^{1}H NMR studies

A solution of FEN (2.0×10^{-4} M) was prepared in 5:1 mixture of D_2O and CD_3OD by serial dilution from the stock solution of FEN. The temperature was adjusted to 298 K. The concentration of DNA varied from 0 to 2.0×10^{-5} M. ^{31}P NMR spectra was recorded at 101.25 MHz on a Bruker Avance DPX 250 MHz (4.7 T). A capillary containing a solution of phosphoric acid (PA) in H_2O was used for the field-frequency lock of NMR spectrometer. In the buffer used and at 298 K, the p2hosphor chemical shift of FEN is 67.86 ppm from PA (external reference). The acquisition parameters (recycle time 8.0 s, flip angle: 90°) were carefully adjusted in order to prevent any saturation of the FEN signal. In the presence of DNA each spectrum needed: 100 scans to give reliable data.

In a typical experiment, the DNA–FEN complex was prepared in $H_2O/D_2O/CD_3OD$ (7:83:10 V:V%) by addition of DNA solution (0.1 mL, 4.0×10^{-5} M) under nitrogen to a septum-capped vial

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